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EXPLORING SEMIOCHEMICAL BASED OVIPOSITION RESPONSE OF *PHLEBOTOMUS ARGENTIPES* (*DIPTERA: PSYCHODIDAE*) TOWARDS PRE-EXISTING COLONY INGREDIENTS

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1. ABSTRACT

This research aimed to evaluate the semio-chemical mediated oviposition responses as well as preference of female *Phlebotomus argentipes* - VL vector in Indian Subcontinent, towards the conspecific eggs and frass i.e, the chief ingredients of pre-existing colony. Through the laboratory based bioassay studies, it was observed that female insects become highly attracted & stimulated to deposit eggs on the surface co-treated with mixtures of aqueous suspension of frass and conspecific eggs (T=2063; 171.92 ± 16.5), as compared to those either treated with aqueous frass filterate (T=1755; 146.25 ± 26.5) or aqueous filtrate of conspecific eggs (T=1514; 126.17 ± 19.2) alone. Comparing the tendency of female insects to oviposit, it was also observed that aqueous frass filtrate (101.625 ± 39.3807) and hexane filtrate of conspecific eggs (T=668; 83.5 ± 15.3) possess high attractancy for the egg deposition by female insect population over the aqueous filterate of conspecific eggs (60.125 ± 20.4341) and hexane filtrate of frass (T=371; 46.3 ± 19.3) respectively.

This observation evidenced that oviposition attractant associated with frass & conspecific eggs (co-working as an oviposition attractant & stimulant for egg deposition by female sandflies) get dissolved in water & organic solvent respectively releasing aroma that positively attract ovipositing female insects towards it & stimulating them for the purpose of egg deposition.

2. KEYWORDS: Semiochemical, *Phlebotomus argentipes*, VL, Oviposition, Attractant, Solvent

3. INTRODUCTION

Pheromones are the chemical-based-substances secreted from organisms' ectodermal gland externally, enabling the conspecifics members to communicate as well as regulate social-response among them (Karlson & Butenandt, 1959). Pheromone mediated responses of non-social insects group, have been the subject of interest for many groups of scientists working on behavioural aspects of insects as they lack protective maternal behaviour. Blood–feeding females' of hematophagous insect of this group are exclusively capable of transmitting disease-agent as well as amplifying its population through oviposited eggs laid by them, after fulfilment of its gut through blood-meal (Seenivasagan & Vijayaraghavan, 2010). The sheer abundance of insects belonging to this category, possess excellent reproductive abilities, complex behaviours associated with searching of enriched blood source for feeding, healthy mating partners for successful breeding & oviposition site for regulation of its generation. Along with these, their ability to

develop resistance against insecticides provides unsatisfactory control of such insects (Logan & Birkett, 2007). Hence evaluation of behavioural-based responses could provide an effective pathway for monitoring them as well as controlling their influence. Various works had been conducted on the behavioural aspects concerning sand flies among the hematophagous insect population, but it is still insufficient as studying oviposition behaviour of sand flies & its extermination for the control of vector as well as disease, fairly remained a neglected aspect (Feliciangeli, 2004). Many relevant work concerning the pheromone & semiochemical mediated behaviour of new world sand fly had been reported. Morton and Ward (1989), Nigam and Ward (1991) found that different populations of *L. longipalpis* showed preferences for mate choice to a conspecific pheromone. Female *L. longipalpis* uses a range of semiochemical cues provided by the physical and chemical constituent of the oviposition-surface at close range during the suitable site searching programme and gets stimulated to oviposit under the influence of pheromone associated with conspecific eggs that are released on to it by the female sand flies during the oviposition (Dougherty et al.1993). Pheromone also influences proper egg-laying in sufficient amount as well as the larval development (Killick-Kendrick, 1999). The remains of previously used colony used for rearing larvae of sand fly (pre-existing sand fly colony remains) comprising organic material of various sources e.g., vertebrate-faeces, larval rearing medium, frass, etc. (Killick-Kendrick, 1999) serves as an oviposition attractants for the females of *L. longipalpis* and *P. papatasi* (Alves et al. 2003, Dougherty et al. 1993, Dougherty et al. 1995, Elnaiem and Ward 1992, Schlein et al. 1989, Schlein et al. 1990). Dougherty et.al, 1994 with the help of Bio assay demonstrated the semiochemical mediated oviposition attraction of gravid *L. longipalpis*, it was demonstrated that during the location of a suitable oviposition site for egg laying (Dougherty et. al, 1994) and a potential host for blood feeding (Kumar et. al 2012), haematophagous insects mainly use olfactory senses for host location as well as for mating. In our previous work (Kumar et al. 2013), it was demonstrated that the pre- existing colony components containing frass, larval food, dead remains of exuviae, etc, are highly enriched with fertile organic material, releases aroma that strongly act as an ovipositor attractant for mature adult females of *P. argentipes*, stimulating them to lay eggs on the surface containing organic material.

In this chapter, comparative approach was adopted through the bioassay oviposition choice-chamber, for evaluating the semio-chemical mediated oviposition responses of female *P. argentipes* towards the sub additive effect of conspecific eggs and frass i.e., chief ingredients of previously existed sand fly colony. Also, comparative evaluation of responses and stimulation of ovipositing female sand flies towards the chief ingredients of pre-existing colony mixed in polar solvent and non-polar solvent dissolving them completely such that releasing aroma potentially attracts ovipositing female *P. argentipes* for successful egg deposition at oviposition surface was conducted through Y-shaped bioassay olfacto-choice chamber. Present study aimed to strengthen sand fly colony following the evaluation of oviposition preferences based research studies for the purpose of having vast & loop hole-free knowledge regarding the behavioral aspect especially oviposition behaviour of *P. argentipes*, so that this knowledge can successfully be manipulated for monitoring and controlling the target insect using integrated vector management strategies & hence controlling the disease.

4. MATERIALS AND METHODS

For conducting bioassay experiments for the evaluation of semiochemical mediated response towards the chief ingredients of previously existed sandfly colony, & for comparative assessment of behavioural response towards the filtrate of conspecific eggs and frass prepared in the organic solvents affecting oviposition of female *P. argentipes*, the pre-requisites are as follows.

Insects: Experiments conducted with *P. argentipes*- an Indian sub continental Sand flies, laboratory strain, reared at controlled temperature of 28 ± 2º C ; 80 ± 5% RH and 12:12 (L: D) h of photoperiod in a closed insectarium of Rajendra Memorial Research Institute of Medical Sciences (ICMR), Agamkuan, Patna, Bihar, India, by following the protocols described by Modi & Tesh (1983), Kumar et.al. (2011).

Conspecific Eggs: Obtained from the established colony of *P. argentipes*. The eggs were counted under the Stereoscopic Microscope (Carl Zeiss Stereoscopy Microscope, Austria; Model no. 426126) one day before from rearing Hilton pots, for colony maintenance. These were collected in 15 ml Tarson® centrifuge tube containing distilled water, thoroughly mixed & kept overnight for refrigeration (4° C).

Aqueous Filterate of Conspecific Eggs: 100-150 eggs of *P. argentipes*, well-mixed in 5 ml distilled water, allowed to settle-down for 10 minutes at room temperature, filtered using standard medical gauze and transferred to 20 ml vial before use.

Hexane Filterate of Conspecific Eggs: Prepared from the 100-150 eggs of *P. argentipes* thoroughly mixed in 5 ml hexane, allowing eggs to settle down before filtration of supernatant through standard medical gauze, filtrate were preserved at -20^{o}c, by following the protocol for the preparation of non-destructive and exhaustive cold extract of organic molecules produced by the insect (Nigam and Ward 1991, Hamilton et al. 1999c).

Frass: Organically enriched larval food remains containing particulate of hatched eggs, exuviae dead remains of larvae, etc. obtained from the old larval rearing pots of sand fly *P. argentipes* colony at the insectarium of Rajendra Memorial Research Institute of Medical Sciences (ICMR), Agamkuan, Patna, Bihar, India.

Aqueous Filterate of Frass: 2 gm frass, well mixed and stirred manually in 10 ml distilled water, allowed to settle for 10 min. at the room temp, filtered using standard medical gauze and transferred to 20ml vial before use. (Wasserberg & Rowton, 2011)

Hexane Filterate of Frass: 2 gm frass well mixed and stirred manually in 10 ml hexane, allowed to settle for 10 min. at the room temp, filtered using standard medical gauze and transferred to 20ml vial before use.

4.1 Semio-Chemical Mediated Oviposition Response of Female *P. argentipes* towards the Sub Additive Effect of Conspecific Eggs and Frass

The Bio assay oviposition choice chamber sets was prepared for determining the semio-chemical mediated oviposition responses of female *P. argentipes* towards the sub additive effect of conspecific eggs and frass i.e., chief ingredients of previously existed sand fly colony. For this purpose a cylindrical plastic jar of 6 cm (height) × 12 cm (diameter) with 15 holes at its base, a big hole was cut out from the centre of its lid. The solution of plaster of Paris [$(CaSO_4)_2$. ½H_2O] and water in the ratio of 2:3 was prepared and set in its base up to ½ cm and left for about 15 minutes to dry. After complete hardening of POP solution, jar was cleaned with cotton and made ready for its use as a bioassay oviposition pot for confining gravid females.

For the Bioassay study the method of Elnaim, 2011 was adopted. The bioassay set had four scratches on its plastered surface in order to divide it into four equal sized quarters. The corner of each quarters of bioassay sets were treated with distilled water, aqueous filtrate of conspecific eggs, aqueous frass filtrate, and combined mixture of aqueous frass filtrate and aqueous filtrate of conspecific eggs. And hence name allocated for each quarters were

CONTROL, EGGS, FRASS, and EGGS+ FRASS respectively on the basis of surface treatment of oviposition pot. The bioassay oviposition choice chamber set is pictorially represented in Figure 1.

4.1.1 Confinement of Adults for Oviposition

New freshly emerged flies both male and female *of P. argentipes* were collected from the colony and were provided glucose feeding after its emergence. Next morning these flies were provided with blood meal of a rabbit (Kumar et al. 2012). Next day 10 active blood-fed female sand flies were captured from the colony with the help of aspiration technique and were carefully transferred into the experimental oviposition pot through the hole made on its lid, and then cotton-ball were plugged over it as soon as successful insertion of flies gets completed. The plastered surface of oviposition pots, post- confinement were kept on cotton cloth soaked in water for the purpose of regular supply of humidity after providing sugar meal source (cotton soaked in a 10% glucose solution).

The oviposition pots were kept in complete darkness for 5 days in controlled condition of insectorium maintained at 28 ± 2^0 C and 80±5 RH for the purpose of proper oviposition by the sand flies. Every day the pots were observed for egg-laying. From the sixth day of confinement, Post-death of insects, the dead flies on the surface of oviposition pots were visually counted, & then were removed carefully with the help of needle such that eggs remained undisturbed at their position. Then these pots were observed under the stereoscopic microscope (Carl Zeiss Stereoscopy Microscope, Austria; Model no. - 426126) for the purpose of counting eggs laid by female population at each quarter of plaster surface of experimental oviposition pot. The data for dead insects as well as egg deposited by them at the surface of oviposition pots were recorded & on that basis comparison were made.

Twelve replicates of experiments were conducted under the identical conditions in order to clearly understand the most favourable component among the old colony, remains viz., frass and conspecific eggs attracting the ovipositing female *P. argentipes* towards it & stimulating them for successful egg deposition. Fresh batches of insects for each replicates of bioassay were used to avoid saturation of the insect chemoreception.

4.2 Comparative Assessment of Behavioral Responses towards the Filtrate of Conspecific Eggs and Frass Prepared in the Organic Solvents Affecting Oviposition in Female *P. argentipes*

In a way to identify the polarity of solvent dissolving the colony component releasing aroma that potentially attracts ovipositing female *P. argentipes* for successful egg deposition at oviposition surface, a comparative response of ovipositing female sand flies for egg deposition towards the chief ingredients of pre-existing colony mixed in polar solvent and non-polar solvent was studied. For this purpose, frass & conspecific eggs filtrate was prepared in water as well as hexane medium representing polar and non polar organic solvent extract of frass as well as conspecific eggs respectively (as explained earlier) to be applied on the filter paper for the surface of oviposition pots (OP) & arranged in Y-shaped bioassay olfacto-choice chamber as, described in upcoming sections & pictorially represented in Figure 2.

4.2.1 Towards the Surface Treated with Aqueous Filtrate of Frass and Aqueous Filtrate of Conspecific Eggs

To evaluate the responsiveness of ovipositing female *P. argentipes*, Y-shaped bioassay olfacto choice chamber set was prepared, comprising 3 oviposition pots, 7 cm ×7 cm (height ×diameter), (OP 1), (OP 2) and (OP 3) containing filter paper impregnated with distilled water, aqueous filtrate of frass and aqueous filtrate of conspecific eggs for oviposition pot (OP) named as CONTROL, AQUEOUS FILTRATE OF FRASS and AQUEOUS FILTRATE OF EGGS respectively.

The 3 Oviposition Pots (OP) were joined to the insertion chamber (IC) 7 cm ×7 cm (height ×diameter), with the help of 3 joining tubes 14.5cm× 1.5 cm (length × diameter), (JT 1), (JT 2) and (JT 3) respectively.

10 active blood-fed female insects were released in prepared into the insertion chamber (IC) of experimental set through the aspiration technique. The bioassay set were kept on semi wetted cotton cloth for proper moisture and humidity after providing Sugar meal source (cotton soaked in a 10% glucose solution). The sets were kept under similar condition as mentioned in previous experiment for the purpose of proper egg deposition by female flies.

After sixth day of confinement, the dead adult insects on the oviposition surface were visually counted & carefully removed without disturbing the original position of eggs in the ovipositing pots. Then the oviposition pots were observed under the stereoscopic microscope for the purpose of counting the eggs deposited by female individuals, similarly as mentioned earlier. The data for dead insects as well as egg deposited by them at the surface of oviposition pots were recorded and comparisons were made on that basis. Record of dead insects & eggs deposited by them at the intermediate joining tubes (JT) were omitted for the purpose of accuracy of result.

The oviposition behavioural responses of Female *P. argentipes* to the aqueous filtrate of frass and conspecific eggs were demonstrated for confirmation by repeating same procedure for 8 times. Each sets of replicates started with fresh batch of female insects.

4.2.2 Towards the Surface Treated with Hexane Filtrate of Frass and Hexane Filtrate of Conspecific Eggs

A similar approach was made through Y-shaped bioassay olfacto-choice chamber for comparative evaluation of responsiveness of ovipositing female *P. argentipes* towards hexane filtrate of conspecific eggs versus hexane filtrate of frass. For this purpose similar experimental Y-shaped bioassay olfacto choice chamber set-up were arranged, comprising 3 oviposition pots, (OP 1), (OP 2) and (OP 3) containing filter paper impregnated with distilled water, hexane filtrate of frass and hexane filtrate of conspecific eggs for oviposition pot (OP) named as CONTROL, HEXANE FILTRATE OF FRASS and HEXANE FILTRATE OF EGGS respectively joined to the insertion chamber (IC) with the help of 3 joining tubes (JT 1), (JT 2) and (JT 3) respectively**.** Protocol for the confinement of adult insect for the oviposition as well as observing the no. of eggs deposited by them, remained same as described earlier in sections 4.1.1 & 4.2.1.

Eight replicates of experiments were conducted under the identical conditions in order to clearly understand the nature of organic solvent on the basis of polarity dissolving the colony component releasing aroma that potentially attracts ovipositing female *P. argentipes* for successful egg deposition at oviposition surface. For each replicates of bioassay, fresh batches of insects were used to avoid saturation of the insect chemoreception.

On the basis of obtained data, a comparative analysis was conducted between the No. of dead female insects & no. of eggs laid by them at the surface of oviposition pots (OP).

5. RESULTS

Phlebotomus Argentipes females found more attractive towards the combined aroma of frass and conspecific eggs of pre-existing colony for the purpose of egg-deposition as the higher no. of dead insects (33.33%) were counted at the quarter labelled as EGGS+ FRASS. Also, Females laid significantly more eggs (T=2063; 171.92 ± 16.5) on the surface co-treated with mixtures of aqueous frass suspension and aqueous extract of conspecific eggs, as compared to the corners

with aqueous frass filterate (T=1755; 146.25 ± 26.5) or aqueous filtrate of conspecific eggs (T=1514; 126.17 ± 19.2) alone. Attractancy of gravid females was found more towards the combined treatment of frass and conspecific eggs on the oviposition surface as represented in Table 1 & Figure 3. This study evidenced the sub additive effect of conspecific eggs and frass filtration (Elnaiem 1992) and proved to be the most attractive for the oviposition response by female *P. argentipes*. Careful observations of data analysis for Semio-chemical mediated oviposition response of female *P. argentipes* towards the sub additive effect of conspecific eggs and frass, we observed that there is very much fluctuation in no. of eggs deposited by the female *P. argentipes* at each quarters of the oviposition choice chamber. One way ANOVA analysis, source of variation between sets (d.f. =3) and mean sum of square of variation (S.S= 29156.19) was calculated for the purpose of observation of variation between the sets of experimental observation. The values of Kurtosis and Skewness for each treatment were far beyond ± 1 as compared to control of replicates of experiment 1. Hence the observation ($F=57.32803$, $P < 0.001$) was found to be significant at 5% level of significance indicating the presence of variation in the observation.

Through the experiments with Y-shaped bioassay olfacto-choice chamber, aqueous filtrate of frass possesses high attractancy (52.83%) over the control (18.86%) as well as aqueous filtrate of conspecific eggs(28.30%) for ovipositing female insect, stimulating them to oviposit (T=813; 101.625 ± 39.3807) as compared to either control (T=349; 46.75 ± 17.36) or aqueous filtrate of conspecific eggs which was recorded as T=481; 60.125 ± 20.4341 (Table 2 & Figure 4), compelling ovipositing female sand flies to deposit eggs on the treated surface. On other hand, female insects were found to be highly attracted towards the surface treated with hexane filtrate of conspecific eggs (52.05%) & stimulated to deposit higher no. of eggs on to it (T=668; 83.5 ± 15.3) as compared to control (17.80%) or hexane filtrate of frass (30.13%) onto which no. of eggs deposition were recorded as T=176; 22 ± 6.8 & T=371; 46.3 ± 19.3 (Table 3 & Figure 5).

This indicate that frass & conspecific eggs i.e., the chief ingredients of pre-existing colony component possessing semiochemical particulate behaves sub-additively for attracting as well as stimulating ovipositing female insects for the purpose of successful egg-deposition. Also through the Y-shaped bioassay olfacto-choice chamber it was confirmed that the frass & conspecific eggs possessing semiochemical particulate get properly dissolved in the polar & nonpolar organic solvent releasing aroma attracting ovipositing female insects as well as stimulating them assured & successful egg-deposition. We observed that data in each replicates contains very much fluctuation in no. of eggs deposited by the female *P. argentipes* at each oviposition pots (OP) experimental sets. Through One way ANOVA analysis, the source of variation between sets (d.f =2) and mean sum of square of variation (S.S= 15345.75) were calculated for observing variation between the experimental replicates. The values of Kurtosis and Skewness for each treatment were beyond ± 1 as compared to control of replicates of experiment 2. Hence the observation ($F=35.04453$, $P < 0.001$) was found to be significant at 5% level of significance indicating the presence of variation in the observation.

6. CONCLUSIONS & DISCUSSIONS

The results obtained from our previous study with individually isolated *P. argentipes* in each experimental pots containing larval rearing medium i.e., larval food (a mixture of rabbit faeces\mixed with sand in equal ratio), dead remains of larvae, exuviae and eggs of sandflies' of pre-existing colony, suggested that larval rearing pots obtained from pre-existing sandfly colony potentially attracts the ovipositing females in controlled conditions (Kumar et.al., 2013).

Wasserberg.et.al, (2011) reported that old colony remains have positive impact on oviposition rate of *L. longipalpis*. The physical and chemical nature of the oviposition surface potentially possess oviposition attractant for gravid sandflies which attracts them towards itself & stimulate them for the purpose of egg deposition (Dougherty.et.al, 1993, Schlein et al. 1989). According to an experiment conducted by Elnaiem & Ward, 1992 demonstrated that *L. longipalpis* preferred the sites with frass and rabbit faeces as its larval rearing medium for the purpose of oviposition. Also in a work Eldin.et.al, 1992 reported that chamber containing frass and larval rearing medium, acts as an attractants for the oviposition of *L. longipalpis*. In a similar experiment performed on *L. longipalpis* and *P. papatasi*, it was found that effect of old sand fly colony remains (frass), conspecific eggs and their combination produces favourable impacts on the oviposition behaviour.

These studies provided us platform for our research on *P. argentipes* and hence, on the basis of experiment conducted in the bioassay oviposition choice chamber, it was concluded that the gravid females become more responsive towards the combined effect of frass and egg filtrate as compared to either whole eggs or frass alone, suggesting the sub additive effect of conspecific eggs as well as frass filtration eliciting attraction for female *P. argentipes* for the egg-deposition. Our result corroborate with the observations of Dougherty et al. (1993) who founded a significant synergistic effect of the combined treatment of eggs pheromone and rabbit food.

In a study conducted by Dia Eldin et.al, (1992) reported that aroma from frass strongly act as an ovipositor attractant for matured adult female sand flies and significantly higher number of eggs were laid in test chamber containing the rabbit faeces than in an untreated control chamber. Significantly higher no. of eggs were laid by *P. papatasi* females in the vicinity of di-ethyl ether extract of conspecific eggs (Srinivasan et.al, 1995) indicating the dissolving of oviposition attractant in organic solvent. Through the comparison based study, with the help of Y-shaped bioassay olfacto-choice chamber, it was evaluated that the frass & conspecific eggs possessing semiochemical particulate get properly dissolved in water (polar) & hexane (nonpolar) medium releasing aroma that attracts ovipositing female sand flies & stimulating them to. Through the study it can be inferred that attractant associated with frass & conspecific eggs gets dissolved in water being polar solvent & hexane being non-polar solvent respectively, compelling ovipositing female sandflies oviposit on the treated surface.

Though, the detection/ identification of chemicals associated with semiochemical releasing colony component involved in the process of attracting & stimulating ovipositing female *P. argentipes* is a big challenge before the scientist to explore but work of exploring the role of synthetic pheromones as a potential sand fly control strategy is almost begun.

7. ACKNOWLEDGEMENTS

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9. APPENDICES

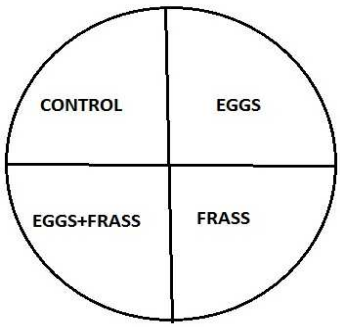

Figure 1: Pictorial Representation of Bio Assay Oviposition Choice Chamber for Demonstrating Semio-Chemical Responses by Female *P. argentipes* towards the Sub Additive Effect of Conspecific Eggs and Frass

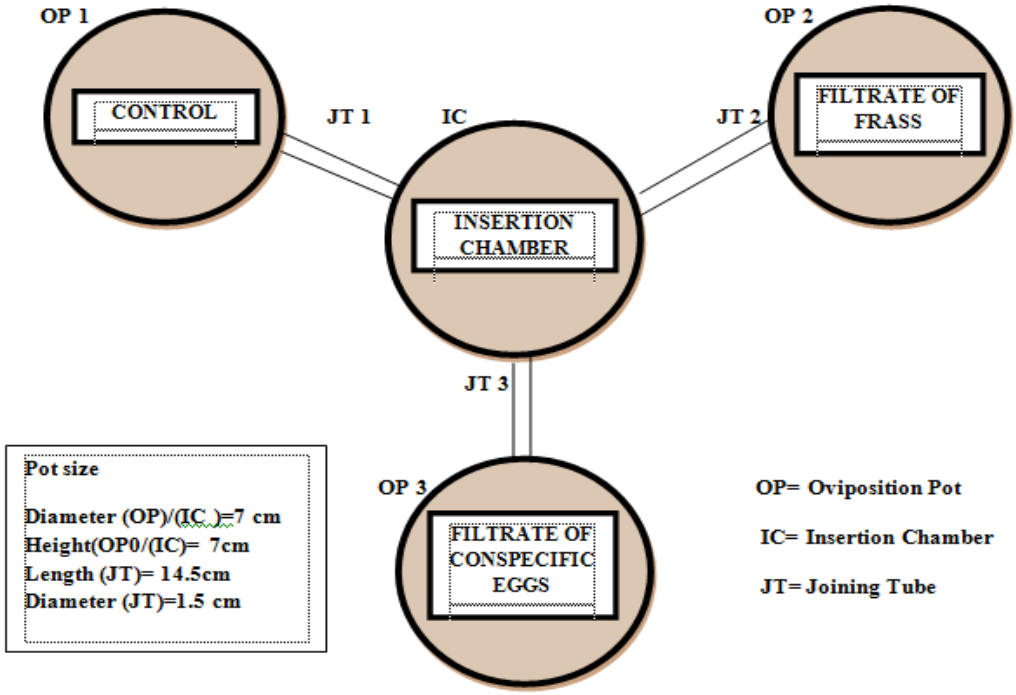

Figure 2: Schematic Arrangements of Oviposition Pots (OP) & Insertion Chamber (IC) in Y-Shaped Bioassay Olfacto-Choice Chamber for Comparative Assessment of Behavioral Responses towards the Filtrate of Conspecific Eggs and Frass Prepared in the Organic Solvents Affecting Oviposition in Female *P. argentipes*

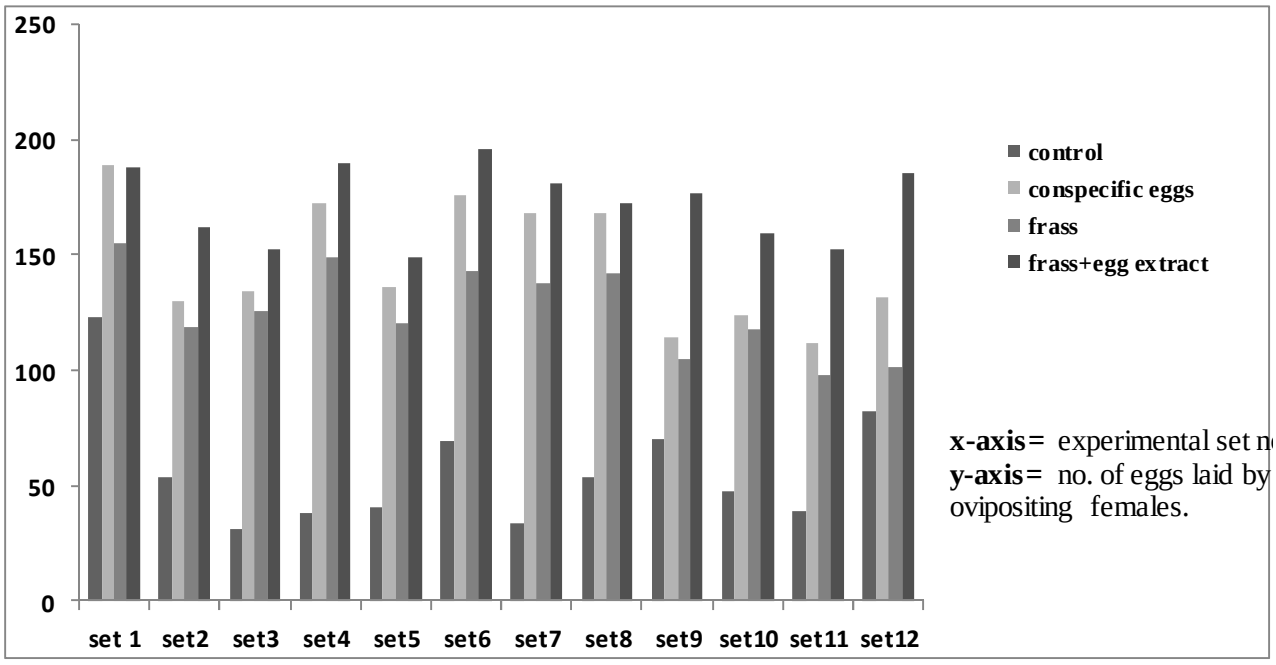

Figure 3: Graphical Analysis of No. of Egg Oviposited by Female Sand Flies at Each Corners of Each Replicates of Bioassay Oviposition Choice Chamber Demonstrating Semio-Chemical Responses of Female *P. argentipes* towards the Sub Additive Effect of Conspecific Eggs and Frass

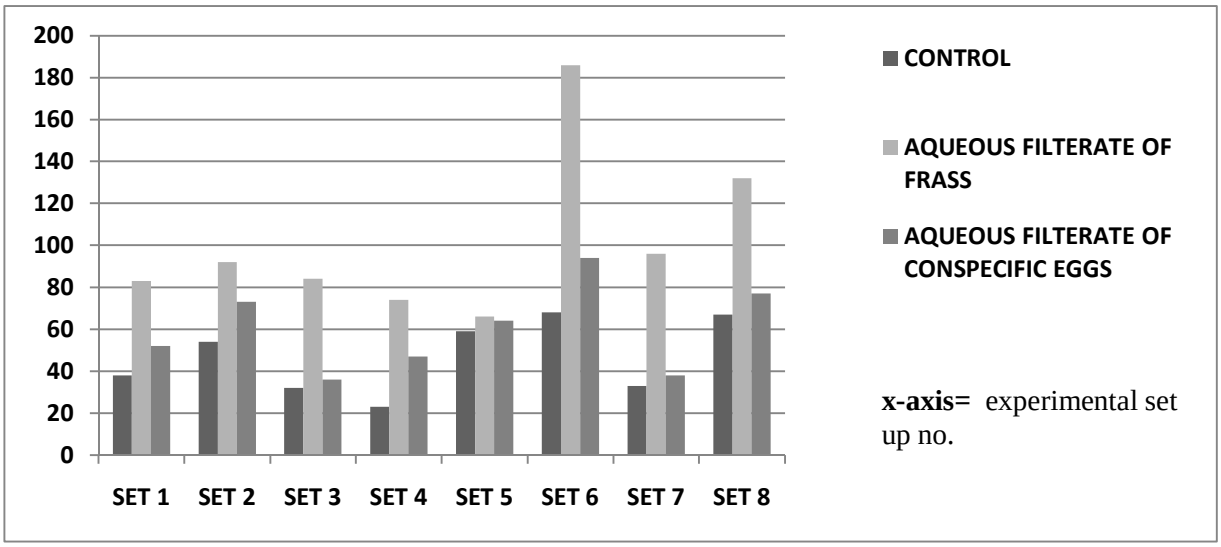

Figure 4: Graphical Analysis of No. of Egg Deposited by Female Sand Flies at Each Oviposition Pots of Each Replicates of Y-Shaped Bioassay Olfacto-Choice Chamber Demonstrating Comparative Response of Female *P. argentipes* towards the Surface Treated with Aqueous Filtrate of Frass and Aqueous Filtrate of Conspecific Eggs

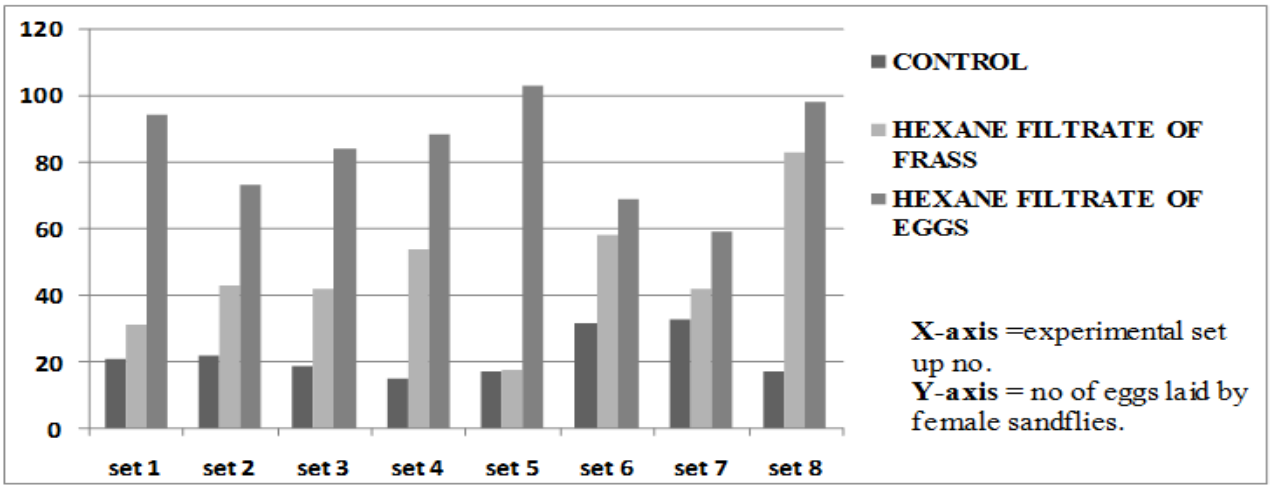

Figure 5*:* Graphical Analysis of No. of Egg Deposited by Female Sand Flies at Each Oviposition Pots of Each Replicates of Y-Shaped Bioassay Olfacto-Choice Chamber Demonstrating Comparative Response of Female *P. argentipes* towards the Surface Treated with Aqueous Filtrate of Frass and Aqueous Filtrate of Conspecific Eggs

Table 1: Data Analysis for Semio-Chemical Mediated Oviposition Response of Female *P. argentipes* towards the Sub Additive Effect of Conspecific Eggs and Frass at Each Corner of Replicates of Bioassay Oviposition Choice Chamber

	Total No. of Adult Insects at Each Corners of Oviposition Pots	Attractancy % of Female Flies	Total no. of Eggs Laid by Female Flies	Mean ± St. Deviation Egg Deposition	Median Egg Deposition	Kurtosis	Skewness
CONTROL	11	16.66	683	56.92 ± 26.1	51	2.859258	1.590675
FRASS	18	27.27	1755	146.25 ± 26.5	135	-1.54029	0.275665
EGGS	15	22.72	1514	126.17 ± 19.2	123	-1.29393	-0.04073
EGGS+FRASS	22	33.33	2063	171.92 ± 16.5	174.5	-1.59129	-0.09082

Table 2: Data Analysis for Comparative Assessment of Behavioral Responses of Female *P. argentipes* towards the Surface of Oviposition Pots (OP) of Y-Shaped Bioassay Olfacto-Choice Chamber Treated with Aqueous Filtrate of Frass and Aqueous Filtrate of Conspecific Eggs for Oviposition

	Total No. of of Adult Insects in OP	Attractancy % of Female Flies	Total No. of Eggs Deposited by Female Flies	Mean ± St. Deviation Egg Deposition	Median	Kurtosis	Skewness
CONTROL	10	18.86	349	46.75±17.36	46	-1.86873	0.008862
AQUEOUS FILTRATE OF FRASS	28	52.83	813	101.625 ± 39.3807	88	2.791212	1.713488
AQUEOUS FILTRATE OF CONSPECIFIC EGGS	15	28.30	481	60.125 ± 20.43413	58	-0.89234	0.406317

Table 3: Data Analysis for Comparative Assessment of Behavioral Responses of Female *P. argentipes* towards the Surface of Oviposition Pots (OP) of Y-Shaped Bioassay Olfacto-Choice Chamber Treated with Hexane Filtrate of Frass and Hexane Filtrate of Conspecific Eggs for Oviposition

	Total No. of Adult Insects in OP	Attractancy % of Female Flies	Total No. of Eggs Laid By Female Flies	Mean ± St. Deviation Egg Deposition	Median Egg Deposition	Kurtosis	Skewness
CONTROL	13	17.80	176	22 ± 6.8	20	-0.52274	1.006266
HEXANE EXTRACT OF FRASS	22	30.13	371	46.3 ± 19.3	42.5	1.254443	0.641166
HEXANE EXTRACT OF EGGS	38	52.05	668	83.5 ± 15.3	86	-1.04907	-0.38173